

Amendments to the Specifications:

Please replace the paragraph starting at page 4, line 4 from bottom of the specification with the following amended paragraph:

The non-TolA polypeptide of the fusion polypeptide may be human BCL-XL (SEQ ID NO: 62, SWISSPROT Accession No. B47537). The fusion polypeptide with human BCL-XL (SEQ ID NO: 62) may comprise the amino acid sequence of SEQ ID NO: 14 or SEQ ID NO: 15. As shown in Example 2 below, large amounts of BCL-XL (SEQ ID NO: 62, an important protein in apoptosis and cancer research) can be generated by expression as a TolAIII fusion polypeptide.

Please replace the paragraph starting at page 17 line 9 from bottom of the specification with the following amended paragraph:

A DNA fragment encoding BCL-XL (SEQ ID NO: 62) was amplified by PCR from the plasmid pETBCLXL using the oligonucleotides SenseBCL-STU (5'-TTT TTT AGG CCT TCT CAG AGC AAC CGG GAG-3'; SEQ ID NO: 60) and Mlu-BCL-Rev (5'-TTT TAC GCG TTC ATT TCC GAC TGA AGA G-3'; SEQ ID NO: 61). BCL-XL (SEQ ID NO: 62) was introduced into pTOLT plasmid using Stu I and Mlu I restriction sites. The final plasmid was named as a pTOLT-BCLXL (FIG. 7) and DNA sequencing of this plasmid showed that BCL-XL (SEQ ID NO: 62) encoding DNA fragment was correctly inserted.

Please replace the paragraph starting at page 18, line 1 of the specification with the following amended paragraph:

BCL-XL (SEQ ID NO: 62) protein was expressed in an E. coli BL21 DE3 (pLysE) strain. The strain was transformed with plasmid and grown on LB plates with ampicillin (200 µg/ml) and chloramphenicol (35 µg/ml) selection. 5 ml of LB medium with antibiotics was inoculated with single colony and grown overnight at 37°C. A 5 ml overnight culture was introduced into 500 ml of LB medium in 2 liter flasks containing

ampicillin and chloramphenicol. Bacteria were grown until OD₆₀₀: 0.8 and induced by addition of final concentration 1 mM IPTG then grown for additional 3 hours. Cells were harvested and resuspended in 20 mM phosphate, 300 mM NaCl, pH: 8.0 buffer containing RNase, DNase, PMSF (1 mM) and Benzamidine (1 mM). The cells were lysed by French press and the supernatant was obtained by ultra-centrifugation at 40 000 rpm for 1 h. The N-terminal 6X Histidine-tag (SEQ ID NO: 8) facilitated purification of the Tol-BCL fusion by means of Ni-NTA affinity column. The fusion protein was washed onto the column with 20 mM phosphate, 300 mM NaCl, pH: 8.0, buffer, additionally washed with the same buffer containing 50 mM imidazole and eluted in 300 mM imidazole, pH 7.0. The expression of fusion protein was analysed by SDS-PAGE (FIG. 8) and concentration of protein was determined by UV absorption at 280 nm.

Please replace the paragraph starting at page 18, line 19 of the specification with the following amended paragraph:

20 mg of TolA-BCL fusion was incubated in 20 ml of cleavage buffer at 4 °C. for 4 h. Cleavage buffer contains 50 mM Tris-HCl, 150 mM NaCl, 2.5 mM CaCl₂, 5 mM DTT and Thrombin (1Unit of thrombin (Sigma)/mg of fused protein). The released protein was recovered applying overnight dialysed cleavage mixture to a Ni-NTA column. After unbound protein was washed from the column, remains of the BCL-XL (SEQ ID NO: 62) protein was washed by 2 M NaCl. All flow through and washes were collected and analysed by SDS-PAGE (FIG. 9). The protein yields were calculated after thrombin cleavage using UV absorbance at 280 nm.

Please replace the paragraph starting at page 19, line 16 of the specification with the following amended paragraph:

Ten proteins were tested in order to check the suitability of pTol expression system for expression and preparation of other proteins (see Example 1, Table 1, and Example 2). These were different parts and domains of colicin N (TolA binding box (peptide of amino acids 40-76), deletion mutant of T-domain (Δ 10) and R domain), representing prokaryotic proteins. Human phospholipase A.sub.2, pore-forming protein

from sea anemone equinatoxin II, nucleotide binding domain 1 (NBD1) of human cystic fibrosis transmembrane conductance regulator (CFTR), human mitochondrial pyruvate dehydrogenase kinase 2 (PDK2) and BCL-XL (SEQ ID NO: 62) were examples of eukaryotic proteins. Transmembrane proteins were represented by BcrC, a component of bacitracin resistance system from *B. licheniformis*, and transmembrane domain 1 (TM1) of human CFTR. Proteins chosen represent variations in size (app. 4.4 of colicin 40-76 kDa vs. 44 kDa of PDK2), genetic code (prokaryotic vs. eukaryotic proteins), protein location (soluble vs. membrane), and disulphide content (PLA₂, 7 disulphides vs. equinatoxin, none). Fusion proteins were expressed at high proportion in *E. coli* using pTol system (FIG. 4). Again, the expression was as high as 40% in some cases, but the average was around 20-25% (see FIGS. 4B and C bottom panels). The only two exceptions were membrane proteins, BcrC and TM1. In this case a band corresponding to their size was lacking from the gel (FIG. 4C). As opposed to expression of TolAIII alone, expression of fusion proteins interferes with the growth of bacteria. In the case of PLA.sub.2 and membrane proteins, TM1 and BcrC, the amount of bacteria at the end of the growth halved in some cases. Interestingly, expression of fusion of PDK2 in bacterial cell had positive effect and there was always slightly more bacteria at the end of the growth (not shown). Some of the bacteria expressing fusions were further fractionated. PDK2 and PLA₂ were expressed as insoluble inclusion bodies. EqtlII and R-domain were found mainly in the insoluble fraction, but some proportion was found also in cytoplasmic fraction (10-25% of expressed proteins) (not shown).

Please replace the paragraph starting at page 21, line 11 of the specification with the following amended paragraph:

We show in Example 2 that BCL-XL (SEQ ID NO: 62), an important protein in apoptosis and cancer research, can be expressed in large quantities as a fusion with TolAIII (see FIG. 8). SDS-PAGE analysis of the TolA-BCL fusion protein revealed a band with an apparent molecular weight of about 35 kD, which is in agreement with the flowing theoretical calculations: